

5 **PLATELET GLYCOPROTEIN IB ALPHA FUSION
POLYPEPTIDES AND METHODS OF USE THEREOF**

RELATED U.S. APPLICATION

This application claims priority to USSN 60/266,838 filed February 6, 2001, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

10 The invention relates to generally to compositions and methods for treating or preventing vascular-associated disorders and more particularly to compositions including platelet glycoprotein IB α -derived polypeptides and methods of using same.

BACKGROUND OF THE INVENTION

15 The deleterious effects of vascular-associated disorders such as stroke, heart attack, and arteroseclerosis are thought to be caused, at least in part, by the inappropriate triggering of a vascular inflammation and repair response. The vascular inflammation and repair response involves adhesive interactions between various cell types normally found freely circulating in blood. Examples of such interactions the interaction that can occur between platelets, leukocytes 20 and the inner wall of blood vessels (*i.e.*, the vascular endothelium). Under conditions of high fluid shear forces, platelets adhere to the endothelium via an interaction between the glycoprotein (GP) Ib-IX-V complex on their surface and von Willebrand factor (vWF) present on exposed vessel subendothelium. In contrast, leukocytes can adhere either directly to activated endothelium or indirectly by first adhering to vWF-immobilized platelets. In both instances, 25 leukocyte cell surface molecules that bind to either the selectins or integrins classes of adhesion receptors mediate these adhesion events. Leukocyte-platelet adhesion is thought to occur, in part, via interaction of the leukocyte surface integrin molecule, Mac1 and the GP1b component of the platelet surface GPIb-IX-V complex.

30 In response to vascular disturbances such as artherosclerotic plaque rupture or mechanical injury, *e.g.*, such as that caused by angioplasty, stent placement, ischemic damage or stenosis,

leukocytes and platelets can accumulate at a vascular lesion site and provide multiple adhesive substrates for one another. This accumulation of leukocytes and platelets lead to the local production of factors including, *e.g.*, mitogens, cytokines and chemokines, causing the further undesirable progression of a vascular disease.

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SUMMARY OF THE INVENTION

The invention is based in part on the discovery of glycoprotein-Ib α -derived fusion proteins that inhibit the adherence of platelets to leukocytes. Accordingly, the glycoprotein-Ib α -derived fusion proteins can be used to treat vascular conditions associated with vascular inflammation, thrombosis, atherosclerosis, and angioplasty-related restenosis. The polypeptides, referred to herein as glycoprotein I α fusion polypeptides.

In one aspect, the invention provides a glycoprotein I α fusion polypeptide that includes a first polypeptide, comprising at least a region of a glycoprotein I α polypeptide, operably linked to a second polypeptide. The second polypeptide is preferably to form a multimer, *e.g.*, a dimer. In preferred embodiments, the second polypeptide comprising at least a region of an immunoglobulin polypeptide. In some embodiments, the fusion protein includes the sequences of GP1b302-Ig (SEQ ID NO:1), Gp1b302/2A-Ig (SEQ ID NO:2), GP1b302/4X-Ig (SEQ ID NO:3), GP1b290 Ig (SEQ ID NO:4), GP1b290/2V-Ig (SEQ ID NO:5), or GP1b290/1A-Ig (SEQ ID NO:6), or a fragment, homolog, analog or derivative thereof. The sequences of these polypeptides are provided below:

20 GP1b302/Ig

MPLLLLLLPSPLHPHPICEVSKVASHLEVNCDKRNLTALPPDLPKDTTILHLSENLLYTFSLA
TLMPYTRLTQLNLDRCELTKLQVDGTLPVLGTLDSHNQLQSLPLLQTLPAUTVLDVSFNRLTS
LPLGALRGLGELQELYLKGNELKTLPPGLLTPTPKLEKLSANNLTELPAAGLLNGLENLDTLLL
QENSLYTIPKGFFGSHLLPFAFLHGNPWLCNCIILYFRRWLQDNAENVYVWKQGVDVKAMTSNVA
25 SVQCDNSDKFPVYKYPGKGCPTLGDEGDTLYDYYPEEDTEGDKVRATRTVVKFPTKARPHTCPP
CPAPEALGAPSVFLFPPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
EQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTIASKAGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:1)

30

GP1b302/2A-Ig

MPLLLLLLPLSPLHPHPICEVSKVASHLEVNCDKRNLTALPPDLPKDTTILHLSENLLYTFSLA
TLMPYTRLTQLNLDRCELTKLQVDGTLPVLTLDLSHNQLQSLPLLGQTLPALTVLDVSFNRLTS
LPLGALRGLGELQELYLKGNELKTLPPGLLTPKLEKLSLANNNLTELPAGLLNGLENLDLLL
QENSLYTIPKGFFGSHLLPFAFLHGNPWLNCIILYFRRWLQDNAENVYVWKQGVVKAMTSNVA
5 SVQCDNSDKFPVYKYPGKGCPTLGDEGDTLYDYYPEEDTEGDKVAATATVVKFPTKARPHTCPP
CPAPEALGAPSVFLLFPPPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:2)

10

GP1b302/4X-Ig

MPLLLLLLPLSPLHPHPICEVSKVASHLEVNCDKRNLTALPPDLPKDTTILHLSENLLYTFSLA
TLMPYTRLTQLNLDRCELTKLQVDGTLPVLTLDLSHNQLQSLPLLGQTLPALTVLDVSFNRLTS
15 LPLGALRGLGELQELYLKGNELKTLPPGLLTPKLEKLSLANNNLTELPAGLLNGLENLDLLL
QENSLYTIPKGFFGSHLLPFAFLHGNPWLNCIILYFRRWLQDNAENVYVWKQVVDVKAVTSNVA
SVQCDNSDKFPVYKYPGKGCPTLGDEGDTLYDYYPEEDTEGDKVAATATVVKFPTKARPHTCPP
CPAPEALGAPSVFLLFPPPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREEM
20 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:3)

GP1b290-Ig

MPLLLLLLPLSPLHPHPICEVSKVASHLEVNCDKRNLTALPPDLPKDTTILHLSENLLYTFSLA
TLMPYTRLTQLNLDRCELTKLQVDGTLPVLTLDLSHNQLQSLPLLGQTLPALTVLDVSFNRLTS
LPLGALRGLGELQELYLKGNELKTLPPGLLTPKLEKLSLANNNLTELPAGLLNGLENLDLLL
QENSLYTIPKGFFGSHLLPFAFLHGNPWLNCIILYFRRWLQDNAENVYVWKQVVDVKAVTSNVA
SVQCDNSDKFPVYKYPGKGCPTLGDEGDTLYDYYPEEDTEGDKVRPHTCPPCAPEALGAPSVF
LFPPPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSL
30 TVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO:4)

GP1b290/2V-Ig

MPLLLLLLPLSPLHPHPICEVSKVASHLEVNCDKRNLTALPPDLPKDTTILHLSENLLYTFSLA
TLMPYTRLTQLNLDRCELTKLQVDGTLPVLTLDLSHNQLQSLPLLGQTLPALTVLDVSFNRLTS
LPLGALRGLGELQELYLKGNELKTLPPGLLTPKLEKLSLANNNLTELPAGLLNGLENLDLLL
QENSLYTIPKGFFGSHLLPFAFLHGNPWLNCIILYFRRWLQDNAENVYVWKQVVDVKAVTSNVA
SVQCDNSDKFPVYKYPGKGCPTLGDEGDTLYDYYPEEDTEGDKVRPHTCPPCAPEALGAPSVF
40 LFPPPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSL
TVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO:5)

GP1b290/1A-Ig

MPLLLLLLPLSPPLHPHPICEVSKVASHLEVNCDKRNLTALPPDLPKDTTILHLSENLLYTFSLA
TLMPYTRLTQLNLDRCETKLQVDGTLPVLGTLDSHNQLQSLPLLGQTLPAUTVLDVSFNRLTS
5 LPLGALRGLGELQELYLKGNELKTLPPGLLTPKLEKLSANNLTELPAGLLNGLENLDLLL
QENSLYTIPKGFFGSHLLPFAFLHGNPWLCNCEILYFRRWLQDNAENVYVWKQGVDVAAMTSNVA
SVQCDNSDKFPVYKYPGKGCPTLGDEGDTLYDYYPEEDTEGDKVRPHTCPCPAPEALGAPSVF
LFPPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
10 TVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPSDIAVEWESENQOPENNYKTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO: 6)

Also provided by the invention is a method of inhibiting leukocyte adhesion to a biological tissue contacting a leukocyte with a glycoprotein Iba α fusion polypeptide according to the invention. The leukocyte is contacted in an amount sufficient to inhibit adherence of the leukocyte and the biological tissue

In another aspect, the invention provides a method of treating a disorder associated with platelet activation. The method includes administering to a subject an effective amount of a glycoprotein Iba α fusion polypeptide.

Also included in the invention is a nucleic acid encoding a glycoprotein Iba α fusion polypeptide, as well as a vector containing glycoprotein Iba α fusion polypeptide-encoding nucleic acids described herein, and a cell containing the vectors or nucleic acids described herein.

Also included in the invention are pharmaceutical compositions that include the glycoprotein Iba α fusion polypeptides, as well as antibodies that specifically recognize the glycoprotein Iba α fusion polypeptides.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an illustration of a coomasie blue stained gel showing the purification of a GP1b302-Ig fusion protein secreted from CHO cells stably transfected with a mammalian expression vector containing a GP1b302-Ig coding region. Lanes 7.9 show protein A eluates containing tryptic fragments (lower band of approximately 38kD). Lane 10 is protein A eluate after gel filtration column (GFC) as described in Figure 2.

FIG. 2 is an illustration of a gel showing the purification of a protein A eluted GP1b302-Ig fusion protein by gel filtration column (GFC). GFC enables separation of upper band (intact fusion protein, lane 4) from lower band (tryptic cleavage fragment, lane 7).

FIG.3 is an illustration of a western blot of conditioned cell culture medium demonstrating the extent of proteolysis for various GP1b-Ig fusion proteins secreted from stability transfected CHO cells.

FIG.4 is a chart depicting an UV spectrum measuring platelet aggregation.

FIG.5 is a chart showing the effect of a single bolus injection of a GPIb290/2V-Ig fusion protein at various concentrations on mean LCX flow patterns during *in vivo* Folts model experiments. Arrow shows time of drug injection.

FIG. 6 is a schematic illustration depicting an injured coronary artery with high fluid shear blood flow.

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DETAILED DESCRIPTION OF THE INVENTION

The invention provides fusion proteins containing glycoprotein Ib α protein-immunoglobulin fusion proteins that are useful for inhibiting adherence of platelets and leukocytes to biological tissues, such as for example the vascular endothelium. The fusion proteins of the invention, or nucleic acids encoding these fusion proteins, can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between an glycoprotein Ib α ligand (such as Von Willebrand Factor, Mac-1, P-selectin or thrombin) and an glycoprotein Ib α protein on the surface of a cell, such as a platelet. Inhibition of binding suppresses glycoprotein Ib α protein -mediated platelet aggregation and associated signal transduction *in vivo*.

The glycoprotein Iba protein-immunoglobulin fusion proteins can be used to modulate the bioavailability of a glycoprotein Iba protein cognate ligand. Inhibition of the glycoprotein Iba protein ligand/glycoprotein Iba protein interaction are useful therapeutically for, *inter alia*, the treatment of vascular inflammation and other vascular disorders associated with platelet activation.

Glycoprotein Iba Fusion Polypeptides

In various aspects the invention provides fusion proteins that include a first polypeptide containing at least a portion of a glycoprotein Iba polypeptide operatively linked to a second polypeptide. As used herein, an glycoprotein Iba "fusion protein" or "chimeric protein" includes at least a portion of a glycoprotein Iba polypeptide operatively linked to a non-glycoprotein Iba polypeptide. An "glycoprotein Iba polypeptide" refers to a polypeptide having an amino acid sequence corresponding to at least a portion of a glycoprotein Iba polypeptide, whereas a "non-glycoprotein Iba polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the glycoprotein Iba protein, e.g., a protein that is different from the glycoprotein Iba polypeptide or fragment and that is derived from the same or a different organism. Within a glycoprotein Iba fusion protein the glycoprotein Iba polypeptide can correspond to all or a portion of an Iba protein.

In one embodiment, a glycoprotein Iba fusion protein comprises at least one biologically active portion of a glycoprotein Iba protein. In another embodiment, a glycoprotein Iba fusion protein comprises at least two biologically active portions of a glycoprotein Iba protein. In yet another embodiment, a glycoprotein Iba fusion protein comprises at least three biologically active portions of a glycoprotein Iba protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the first and second polypeptides are chemically linked (most typically via a covalent bond such as a peptide bond) in a manner that allows for at least one function associated with a glycoprotein Iba polypeptide. When used to refer to nucleic acids encoding a glycoprotein Iba fusion polypeptide, the term operatively linked means that a nucleic acid encoding the glycoprotein Iba polypeptide and the non-glycoprotein Iba polypeptide are fused in-frame to each other. The non-glycoprotein Iba polypeptide can be fused to the N-terminus or C-terminus of the glycoprotein Iba polypeptide.

In a further embodiment, the glycoprotein Iba fusion protein may be linked to one or more additional moieties. For example, the glycoprotein Iba fusion protein may additionally be

linked to a GST fusion protein in which the glycoprotein Ib α fusion protein sequences are fused to the C-terminus of the GST (*i.e.*, glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of glycoprotein Ib α fusion protein.

In another embodiment, the fusion protein includes a heterologous signal sequence
5 (*i.e.*, a polypeptide sequence that is not present in a polypeptide encoded by a glycoprotein Ib α nucleic acid) at its N-terminus. For example, the native glycoprotein Ib α signal sequence can be removed and replaced with a signal sequence from another protein. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of glycoprotein Ib α can be increased through use of a heterologous signal sequence.

10 An chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to
15 avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers.

Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for
20 example, Ausubel *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that encode a fusion moiety (*e.g.*, an Fc region of an immunoglobulin heavy chain). A glycoprotein Ib α encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the immunoglobulin protein.

25 In various embodiments, the glycoprotein Ib α fusion polypeptide includes the amino acid sequence of one or more of SEQ ID NOs: 1-6.

Glycoprotein Ib α fusion polypeptides may exist as oligomers, such as dimers or trimers. Preferably the glycoprotein Ib α fusion polypeptide is a dimer.

The first polypeptide, and/or nucleic acids encoding the first polypeptide, can be
30 constructed using GP Ib α encoding sequences are known in the art and are described in, *e.g.* European Patent Application Publication No. 0 317 278 A2, and Lopez *et al.* 84:5615-19, 1987. Other sources for GP Ib α polypeptides and nucleic acids encoding GP Ib α polypeptides include

GenBank Accession Nos. BAB12038 and AB038516, D85894 and BAA12911, respectively (human sequences), and GenBank Accession No. AAC53320 and U91967, respectively, and are incorporated herein by reference in their entirety.

In some embodiments, the GP Ib α polypeptide moiety is provided as a variant GP Ib α polypeptide having a mutation in the naturally-occurring GP Ib α sequence (wild type) that results in higher affinity (relative to the non-mutated sequence) binding of the GP Ib α polypeptide to a leukocyte cell surface molecule. For example, the mutant polypeptide may bind with higher affinity to Von Willebrand factor (vWF). This increased reactivity, or hyperresponsiveness, can be assessed using low concentrations of ristocetin. Alternately, any other suitable means for determining the reactivity of the polypeptide with vWF can also be utilized to identify polypeptides which are "more" reactive with vWF, *i.e.* more reactive than naturally-occurring wild-type GP Ib α . Examples of GP Ib α polypeptide variants that bind with higher affinity to vWF include GP Ib α variants that include sequence alterations in the hinge region of a GP Ib α polypeptide. The hinge region is defined as the region including residues 220 to 310 and is reported to be a major binding site for vWF within the GP Ib α polypeptide. Mutations in the hinge region include those at residue 233, which in the wild-type GP Ib α encodes glycine. A substitution of valine for glycine 233 is preferred, but other amino acids could also be substituted. A second site for mutation at the hinge region is at residue 239, which in the wild-type GP Ib α encodes methionine. A substitution of valine for glycine 239 is preferred, but other amino acids can also be substituted. In addition, hinge region variants of GP Ib α polypeptides suitable for use in a fusion polypeptide of the invention have mutations at residue both positions 233 and 239. (*see e.g.*, Dong et al., JBC 275:36 27663-27670 (2000)) Thus, the invention includes fusion proteins that have a substitution at position 239, *e.g.*, an M239V substititon of a variant GP Ib α polypeptide. Also within the invention is a fusion protein having a substitution at position 233, *e.g.*, G233V, and a fusion protein that includes a variant GP Ib α polypeptide with positions at both 233 and 239, *e.g.*, a G233V and M239V substitution..

In some embodiments, the GP Ib α polypeptide moiety is provided as a variant GP Ib α polypeptide having mutations in the naturally-occurring GP Ib α sequence (wild type) that results in a GP Ib α sequence more resistant to proteolysis (relative to the non-mutated sequence). Tryptic cleavage sites in the naturally-occurring GP Ib α sequence have been described. (*see e.g.* Titani et al., PNAS 84: 5610-5614. (1987))

In some embodiments, the first polypeptide includes full-length GP Ib α polypeptide.

Alternatively, the first polypeptide comprise less than full-length GP Ib α polypeptide. For example the first polypeptide less than 600 amino acids in length, e.g., less than or equal to 500, 250, 150, 100, 50, or 25 amino acids in length.

5 Examples of a first polypeptide include a polypeptide which includes the amino acid sequence of any of the GP Ib α polypeptide sequences of GP1b302 (SEQ ID NO:7), GP1b302/2A (SEQ ID NO:8) GP1b/4X (SEQ ID NO:9), GP1b290 (SEQ ID NO:10), GB1b290/2V (SEQ ID NO:11) and GB1b290/1A (SEQ ID NO:12).

10 HPICEVSKVASHLEVNCDKRNL TALPPDLPKDTTILHLSENLLYTFSLATLMPYTRLTQLNLD R
CELTKLQVDGTLPV LGTL DLSHNQLQSLPLLGQ TL PALT VLDV SFNRLT S LPLG ALRGL GELQE
LYLKGNELKTLPPG LLPTPKLEKLSL ANNNT LTEL PA GLLNGLENLD TLLQENS LYTI PKG FF
GSHLLPFAFLHGNPWL CNCE ILYFRRWLQDNAENVYVWKQGV DVKA MTSNVASVQCDNSDKFPV
YKYPGKGCP TLGDEGDT DLYDYYPEEDTEGDKVR ATRTVVKFPTKA (SEQ ID NO:7)

15 HPICEVSKVASHLEVNCDKRNL TALPPDLPKDTTILHLSENLLYTFSLATLMPYTRLTQLNLD R
CELTKLQVDGTLPV LGTL DLSHNQLQSLPLLGQ TL PALT VLDV SFNRLT S LPLG ALRGL GELQE
LYLKGNELKTLPPG LLPTPKLEKLSL ANNNT LTEL PA GLLNGLENLD TLLQENS LYTI PKG FF
GSHLLPFAFLHGNPWL CNCE ILYFRRWLQDNAENVYVWKQGV DVKA MTSNVASVQCDNSDKFPV
YKYPGKGCP TLGDEGDT DLYDYYPEEDTEGDKVA ATATVVKFPTKA (SEQ ID NO:8)

20 HPICEVSKVASHLEVNCDKRNL TALPPDLPKDTTILHLSENLLYTFSLATLMPYTRLTQLNLD R
CELTKLQVDGTLPV LGTL DLSHNQLQSLPLLGQ TL PALT VLDV SFNRLT S LPLG ALRGL GELQE
LYLKGNELKTLPPG LLPTPKLEKLSL ANNNT LTEL PA GLLNGLENLD TLLQENS LYTI PKG FF
GSHLLPFAFLHGNPWL CNCE ILYFRRWLQDNAENVYVWKQGV DVKA VTSNVASVQCDNSDKFPV
YKYPGKGCP TLGDEGDT DLYDYYPEEDTEGDKVA ATATVVKFPTKA (SEQ ID NO:9)

25 HPICEVSKVASHLEVNCDKRNL TALPPDLPKDTTILHLSENLLYTFSLATLMPYTRLTQLNLD R
CELTKLQVDGTLPV LGTL DLSHNQLQSLPLLGQ TL PALT VLDV SFNRLT S LPLG ALRGL GELQE
LYLKGNELKTLPPG LLPTPKLEKLSL ANNNT LTEL PA GLLNGLENLD TLLQENS LYTI PKG FF
GSHLLPFAFLHGNPWL CNCE ILYFRRWLQDNAENVYVWKQGV DVKA MTSNVASVQCDNSDKFPV
YKYPGKGCP TLGDEGDT DLYDYYPEEDTEGDKVR (SEQ ID NO:10)

30 HPICEVSKVASHLEVNCDKRNL TALPPDLPKDTTILHLSENLLYTFSLATLMPYTRLTQLNLD R
CELTKLQVDGTLPV LGTL DLSHNQLQSLPLLGQ TL PALT VLDV SFNRLT S LPLG ALRGL GELQE
LYLKGNELKTLPPG LLPTPKLEKLSL ANNNT LTEL PA GLLNGLENLD TLLQENS LYTI PKG FF
GSHLLPFAFLHGNPWL CNCE ILYFRRWLQDNAENVYVWKQGV DVKA VTSNVASVQCDNSDKFPV
YKYPGKGCP TLGDEGDT DLYDYYPEEDTEGDKVR (SEQ ID NO:11)

YKYPGKGCP TLGDEGDT DLYDYYPEEDTEGDKVR (SEQ ID NO:11)

HPICEVSKVASHLEVNC KRNLTALPPDLPKD TILHLSENLLYTFSLATLMPYTRLTQLNLD R
CELT KLQVDGTL PVLGTL DLSHNQLQSLPLLQGTL PALT VLDVS FNR LSLPLG ALRGL GELQE

5 LY LGNE LKTL PPG LLTPK LEK LSL ANN NTEL PA GLN GLEN LD TLL QENS LY TIP KG FF
GSHLLPFAFLHGNPWL CNCE ILYF RRWL QDNA ENV YVWK QGV DVAAM TSNV ASV QCD NSDK FP V
YKYPGKGCP TLGDEGDT DLYDYYPEEDTEGDKVR (SEQ ID NO:12)

A signal peptide that can be included in the fusion protein is MPLLLLLL SPLHP (SEQ ID
10 NO:13). If desired, one or more amino acids can additionally be inserted between the first polypeptide
moiety comprising the GP Ib α moiety and the second polypeptide moiety.

The second polypeptide is preferably soluble. In some embodiments, the second
polypeptide enhances the half-life, (e.g., the serum half-life) of the linked polypeptide. In some
embodiments, the second polypeptide includes a sequence that facilitates association of the
15 fusion polypeptide with a second GP Ib α polypeptide. In preferred embodiments, the second
polypeptide includes at least a region of an immunoglobulin polypeptide. Immunoglobulin
fusion polypeptide are known in the art and are described in e.g., US Patent Nos. 5,516,964;
5,225,538; 5,428,130; 5,514,582; 5,714,147; and 5,455,165.

In some embodiments, the second polypeptide comprises a full-length immunoglobulin
20 polypeptide. Alternatively, the second polypeptide comprise less than full-length
immunoglobulin polypeptide, e.g., a heavy chain, light chain, Fab, Fab₂, Fv, or Fc. Preferably,
the second polypeptide includes the heavy chain of an immunoglobulin polypeptide. More
preferably the second polypeptide includes the Fc region of an immunoglobulin polypeptide.

In another aspect of the invention the second polypeptide has less effector function than
25 the effector function of a Fc region of a wild-type immunoglobulin heavy chain. Fc effector
function includes for example, Fc receptor binding, complement fixation and T cell depleting
activity. (*see for example*, US Patent No. 6,136,310) Methods of assaying T cell depleting
activity, Fc effector function, and antibody stability are known in the art. In one embodiment the
activity, Fc effector function, and antibody stability are known in the art. In one embodiment the
second polypeptide has low or no affinity for the Fc receptor. In an alternative embodiment, the
30 second polypeptide has low or no affinity for complement protein C1q.

A preferred second polypeptide sequence includes the amino acid sequence of SEQ ID
NO: 12. This sequence includes a Fc region. Underlined amino acids are those that differ from
the amino acid found in the corresponding position of the wild-type immunoglobulin sequence:

HTCPPCPAPEALGAPSVLFPPPKDTLMISRTPEVTCVVVDVSHEDEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:14)

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DNA sequences encoding fusion polypeptides of SEQ ID NOS:1-6 are disclosed below as sequences SEQ ID NOS:15-20, respectively:

GP1b302-Ig nucleotide sequence

10 atgcctctcctccttgcgtccgtctgccaagccccttacaccccccacccatctgtgagg
ctccaaagtggccagccacctaagaagtgaactgtgacaagagagaatctgacagcgctgcctccag
acctgccgaaagacacaaccatcctccacccgtgagaaccccttcgtacaccccttcctccggca
accctgatgccttacactcgccctactcagctgaaccttagatagggtgcgagctcaccaagctcca
15 ggtcgatgggacgctgcaggctgtggggaccctggatctatcccacaatcagctgcaaagcctgc
ccttgcttagggcagacactgcctgtctaccgtcctggacgtctccttaaccggctgacccctcg
ctgcctcttggtgcctgcgtggcttggcgaactccaagagactctacccgttggaaaggcaatgagct
gaagaccctgcggccagggtctctgacgcccacaccacgtggagaagctcagttggctaaaca
acaacttgacttagctcccgctgggtcttggataatgggcttgggtcccacccctgccttgc
20 caagagaactcgctgtatacaataccaaagggtttttgggtcccacccctgccttgc
tctccacgggaaccctggttatgcaactgtgagatcccttatccgtcgtggctgcaggaca
atgctgaaaatgtctacgtatggaaagcaagggtggacgtcaaggccatgaccccttaacgtggcc
agtgtgcagtgtgacaattcagacaagttccgttacaaataccaggaaagggtggcc
25 ccttgggtatgaaagggtgacacagacccatatgattactaccagaagaggacactgaggccgata
aggtgcgtgccacaaggactgtggtcaagttcccccacccaaagcgcggccgcacacatgcccaccc
tgcccagcaccctgaaggccctggggcaccgtcagttcccttcccccacccaaacccaaaggacac
cctcatgatctccggacccctgagggtcacatgcgtgggtggacgtgagccaccaagaccctg
30 aggtcaagttcaactggtagctggacggcggtggaggtgcataatgcaagacaaagccgcggag
gagcagtacaacacgcacgtaccgtgtggtagcgtccctcaccgtcctgcaccaggactggctgaa
tggcaaggagtacaagtgcaggtctccaaacaaaggccctccaggccatccggaggagatg
ccaaaggccaaaggccagggcggaaaccacagggttacaccctggccatccggaggagatg
35 accaagaaccaggcagccctgacccctgacccctggtcaaaggcttccatccaggacatgc
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctccgtgactccgac
gccccttccctctacagcaagctcaccgtggacaagagcagggtggcagcaggaaacgttcc
tcattgtccgtatgcatgaggctctgcacaaccactacacgcagaagaggccctccctgttcc
ggtaaa (SEQ ID NO:15)

35

GP1b302/2A-Ig nucleotide sequence

40 atgcctctcctccttgcgtccgtctgccaagccccttacaccccccacccatctgtgagg
ctccaaagtggccagccacctaagaagtgaactgtgacaagagagaatctgacagcgctgcctccag
acctgccgaaagacacaaccatcctccacccgtgagaaccccttcgtacaccccttcctccggca
accctgatgccttacactcgccctactcagctgaaccttagatagggtgcgagctcaccaagctcca
45 ggtcgatgggacgctgcaggctgtggggaccctggatctatcccacaatcagctgcaaagcctgc
ccttgcttagggcagacactgcctgtctaccgtcctggacgtctccttcaaccggctgacccctcg
ctgcctcttggtgcctgcgtggcttggcaactccaagagactctacccgttggaaaggcaatgagct
gaagaccctgcggccagggtccctgacccacaccacgtggagaagctcagttggctaaaca
acaacttgacttagctcccgctgggtcttggataataccaaagggtttttgggtcccacccctgc
50 tctccacgggaaccctggttatgcaactgtgagatcccttatccgtcgtggctgcaggaca
atgctgaaaatgtctacgtatggaaagcaagggtggacgtcaaggccatgaccccttaacgtggcc

agtgtgcagtgtgacaattcagacaagttccgtctacaaatacccaggaaagggggtccccac
ccttggatgaagggtgacacagacatgattactaccagaagaggacactgagggcgata
aggtggctgccacagcgactgtggcaagttccccaccaaagcgcggccgcacacatgcccac
tgcccagcacctaagccctggggcaccgtcagtcttccttccccaaaacccaaggacac
5 cctcatgatctccggacccctgagggtcacatgcgtgggtggacgtgagccacgaagaccc
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gagcagtacaacacgcacgtaccgtgtggcagcgtcctcaccgtcctgcaccaggactggctgaa
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ccaaagccaaaggcgagccccgagaaccacagggttacaccctgccccatccgggaggagatg
10 accaagaaccaggtcagcctgacccctggtaaaggcttcatccagcgcacatgcgcgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctccgtgtggactccgacg
gccccttcttcctacagcaagctcaccgtggacaagagcaggtggcagcagggaaacgtctc
tcatgctccgtgtgcatgaggctctgcacaaccactacacgcagaagagccctccctgtctcc
ggtaaa (SEQ ID NO:16)

15

GP1b302/4X-Ig nucleotide sequence

atgcctctcccttgcgtcttgcgtccacccccacccatctgtgaggt
ctccaaagtggccagccacctagaagtgaactgtgacaagagagaatctgacagcgcgcctccag
acctgcccggaaagacacaaccatctccacccgtgatcttgcgttccatccctggca
20 accctgatgccttacactgcctcactcagctgaaccttagataggcgcagtcaccaagctca
ggtcgatgggacgcgtccagggtgtgggaccctggatctatcccacaatcagctgcaagcctgc
ccttgcgttagggcagacactgcctgtctcaccgtcctggacgtctccctcaaccggctgaccc
ctgccttgcgtggccctgcgtggcttggcaactccaagagacttacccatcggcaatgagct
gaagaccctgcggccagggttgcgttgcgtccacccacaccaagctggagaagctcagtc
25 acaacttgactgagctcccgctgggttgcgttgcgttgcgttgcgttgcgttgcgttgcgtt
caagagaactcgctgtataacaataccaaagggttttgggtcccacccctgcgttgcgttgcgtt
tctccacggaaaccctggttatgcaactgtgagatccctatttcgctgcgtggctgcaggaca
atgctaaaatgtctacgtatggaaagcaagggtggacgtcaaggccgtgacctctaactggcc
30 agtgcgcgtgtgacaattcagacaagttccgttacaaataccaggaaagggtggcc
ccttggatgaagggtgacacagacatgattactaccagaagaggacactgagggcgata
aggtggctccacagcgactgtggcaagttccccaccaaagcgcggccgcacacatgcccac
tgcccagcacctaagccctggggcaccgtcagtcttccttccccaaaacccaaggacac
cctcatgatctccggacccctgagggtcacatgcgtgggtggacgtgagccacgaagaccc
35 aggtcaagttcaactggtaactggacggcggtgcataatgcaagacaaagccgcggag
gagcagtacaacacgcacgtaccgtgtggcagcgttccatccgtcctgcaccaggactggctgaa
tggcaaggagtacaaggtaactggcaagggttccaaacaaaggccctcccaactcgagaaaaccatct
ccaaagccaaaggcgagccccgagaaccacagggttacaccctgccccatccgggaggagatg
40 accaagaaccaggtcagcctgacccctggtaaaggcttcatccagcgcacatgcgcgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctccgtgtggactccgacg
gccccttcttcctacagcaagctcaccgtggacaagagcaggtggcagcagggaaacgtctc
tcatgctccgtgtgcatgaggctctgcacaaccactacacgcagaagagccctccctgtctcc
ggtaaa (SEQ ID NO:17)

GP1b290-Ig nucleotide sequence

45 atgcctctcccttgcgtcttgcgtccacccccacccatctgtgaggt
ctccaaagtggccagccacctagaagtgaactgtgacaagagagaatctgacagcgcgcctccag
acctgcccggaaagacacaaccatctccacccgtgatcttgcgttccatccctggca
accctgatgccttacactgcctcactcagctgaaccttagataggcgcagtcaccaagctca
ggtcgatgggacgcgtccagggtgtgggaccctggatctatcccacaatcagctgcaagcctgc
50 cttgcgttagggcagacactgcctgtctcaccgtcctggacgtctccctcaaccggctgaccc
ctgccttgcgtggccctgcgtggcttggcaactccaagagacttacccatcggcaatgagct
gaagaccctgcggccagggttgcgttgcgtccacccacaccaagctggagaagctcagtc
tggctaa

GP1b290/2V-Ig nucleotide sequence

20 atgcctctcctcccttgcgtgcctgtgccaagcccccttacacccccaccccatctgtgagg
ctccaaagtggccagccacctaagaagtgaactgtgacaagaggaatctgacagcgctgcctccag
acctgccaaagacacaaccatcctcacctgagtgagaacctcctgtacacctctccctggca
accctgatgccttacactcgctcactcagctgaaccttagataggtgcgagctaccaagctcca
ggtcgatgggacgctgcccagtgctgggaccctggatctatcccacaatcagctgcaaagcctgc
ccttgcttagggcagacactgcctgcctcaccgtcctggacgtctccttcaaccggctgacctcg
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acaacttgcgtgagctcccgctggctcctgaatgggctggagaatctcgacaccctctcctc
caagagaactcgctgtataacaataccaaagggtttttgggtcccacctcgcctttgctt
30 tctccacgggaaccctggtatgcaactgtgagatcctctatttcgtcgctggctgcaggaca
atgctaaaatgtctacgtatggaagaagcaagtgggtggacgtcaaggccgtacactctaactggcc
agtgtgcagtgtgacaattcagacaagttcccgctacaataccaggaaagggtgccccac
ccttggtgatgaaggtgacacagacatatgattactaccagaagaggacactgaggggcgata
aggtgcggccgcacacatgcccaccgtgcccagcacctgaagccctggggcacccgtcagtc
35 ctcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggt
ggtggacgtgagccacgaagaccctgaggtcaagttcaactggtaactgtggacggcgtggaggtgc
ataatgccaagacaaaagccgcggagggagcagttacaacacgcacgtaccgttggtcagcgtc
accgtcctgcaccaggactggctgaatggcaaggagtacaagtgcacaggctccaacaaaaggcc
40 cccagtcggccatcgagaaaaccatctccaaaggccaaaggcagccccgagaaccacagggttaca
ccctgccccccatcccgaggagatgaccaagaaccagggtcagcctgacctgcctggtaaaggc
ttctatcccgacatgcgtggagtgggagagcaatggcagccggagaacaactacaagac
cacgcctcccggtctggactccgacggcccttctccttacagcaagctcaccgtggacaaga
gcaggtggcagcagggaaacgtttctcatgctccgtatgcacatgaggctctgcacaaccactac
acgcagaagacgcctccctgtctccggtaaa (SEQ ID NO:19)

45

GP1b290/1A-Ig nucleotide sequence

50 atgccttcctccttgcgtccgtccgtccggccatctgtgaggctccaaagtggccagccacctaagaactgtgacaagaggaatctgacagcgctgcctccagacctgccgaaagacacaaccatcctccacctgagtgagtgagaacccctgtacacccctccctggcacccctgatgccttacactcgccctactcagctgaaccttagataggtgcgagctcaccaagctccaggtcgatgggacgcgtgccagtgctgggaccctggatctatcccacaatcagctgcaaagcctgccttqctaqqqcaqacactqctqctcaccqtcqacqtctcccttaaccggctgacctcg

ctgccttggccctcggtttggcgaactccaagagactaacatggcaatgagct
gaagaccctgccccagggttcctgacgcccacaccaagctggagaagtcagtcggctaaca
acaacttgactgagctcccgctggcttcataatccaaagggtttttgggtcccaccccttc
caagagaactcgctgtatacaataccaaagggtttttgggtcccaccccttc
5 tctccacggaaaccctggttatgcaactgtgagatcttcatggcgtggcagagaca
atgctgaaaatgtctacgtatggaaagggtgtggacgtcgccatgacatcttaacgtggcc
agtgtcagtgacaattcagacaagttccgttacaaataccaggaaagggtggcc
ccttgggtatgaaagggtgacacagacatatgattactaccagaagaggacactgaggcgata
aggtcgccgcacacatgcccacccgtgcccagcacactgaaggccctggggcacccgtcagtc
10 ctctccccccaaaaccaaggacaccctcatgatctccggaccctgaggtcacatgcgttgt
ggtggacgtgagccacgaagaccctgaggtcaagttcaactgttacgtggacggcgtggaggtgc
ataatgccaagacaaagccgcggaggagcagtacaacacgacgttacccgtgtggtcagcgtc
accgtcctgcaccaggactggctgaatggcaaggagatacaagtgcacggatccaaaccc
15 cccagtcggccatcgagaaaaccatctccaaagccaaaggccagccggagaaccacagggttaca
ccctggcccatccggaggagatgaccaagaaccaggctgacccgttacccgttacccgttaca
ttctatcccgacatcgccgtggagtgggagagaatggcagccggagaacaactacaagac
cacgcctcccgctggactccgacggcccttcccttacccgttacccgttaca
gcaggtggcagcaggaaacgtttctcatgctccgtatgcatgaggctgtgcacaaccactac
acgcagaagagccctccctgttccggtaaa (SEQ ID NO:20)

20

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding glycoprotein Iba fusion polypeptides, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type 25 of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon 30 introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the 35 most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the 40 invention in a form suitable for expression of the nucleic acid in a host cell, which means that the

recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that

5 allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 10 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., glycoprotein I $\beta\alpha$ fusion polypeptides, mutant forms of glycoprotein I $\beta\alpha$ fusion polypeptides, etc.).

The recombinant expression vectors of the invention can be designed for expression of 20 glycoprotein I $\beta\alpha$ fusion polypeptides in prokaryotic or eukaryotic cells. For example, glycoprotein I $\beta\alpha$ fusion polypeptides can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant 25 expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, 30 usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (i) to increase expression of recombinant protein; (ii) to increase the solubility of the recombinant protein; and (iii) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site

is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, e.g., Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (see, e.g., Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the glycoprotein Iba fusion polypeptide expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYEPSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, glycoprotein Iba fusion polypeptide can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from

polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, e.g., Chapters 16 and 17 of Sambrook, et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

5 In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, et al., 1987. *Genes Dev.* 1: 268-277),
10 lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8: 729-733) and immunoglobulins (Banerji, et al., 1983. *Cell* 33: 729-740; Queen and Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, et al., 1985. *Science* 230: 912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, e.g., the murine hox promoters (Kessel and Gruss, 1990. *Science* 249: 374-379) and the α -fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

20 The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to NOV glycoprotein Iba fusion polypeptide mRNA. Regulatory sequences operatively linked to a
25 nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are
30 produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see, e.g., Weintraub, et al., "Antisense RNA as a molecular tool for genetic analysis." *Reviews-Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain 5 modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, glycoprotein I β α fusion polypeptides can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or 10 mammalian cells (such as human, Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing 15 foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host 20 cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding glycoprotein I β α fusion polypeptides or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that 25 have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) glycoprotein I β α fusion polypeptides. Accordingly, the invention further provides methods for producing glycoprotein I β α fusion polypeptides using the

host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding glycoprotein Iba fusion polypeptides has been introduced) in a suitable medium such that glycoprotein Iba fusion polypeptides is produced. In another embodiment, the method further comprises isolating 5 glycoprotein Iba fusion polypeptide from the medium or the host cell.

The fusion polypeptides may be isolated and purified in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis or the like. For example, the immunoglobulin fusion proteins may be purified by passing a solution through a column which contains immobilized protein A or protein G which 10 selectively binds the Fc portion of the fusion protein. See, for example, Reis, K. J., et al., *J. Immunol.* 132:3098-3102 (1984); PCT Application, Publication No. WO87/00329. The fusion polypeptide may be eluted by treatment with a chaotropic salt or by elution with aqueous acetic acid (1 M).

Alternatively, fusion polypeptides according to the invention can be chemically 15 synthesized using methods known in the art. Chemical synthesis of polypeptides is described in, e.g., A variety of protein synthesis methods are common in the art, including synthesis using a peptide synthesizer. See, e.g., *Peptide Chemistry, A Practical Textbook*, Bodansky, Ed. Springer-Verlag, 1988; Merrifield, *Science* 232: 241-247 (1986); Barany, et al. *Intl. J. Peptide Protein Res.* 30: 705-739 (1987); Kent, *Ann. Rev. Biochem.* 57:957-989 (1988), and Kaiser, et al. 20 *Science* 243: 187-198 (1989). The polypeptides are purified so that they are substantially free of chemical precursors or other chemicals using standard peptide purification techniques. The language "substantially free of chemical precursors or other chemicals" includes preparations of peptide in which the peptide is separated from chemical precursors or other chemicals that are involved in the synthesis of the peptide. In one embodiment, the language "substantially free of 25 chemical precursors or other chemicals" includes preparations of peptide having less than about 30% (by dry weight) of chemical precursors or non-peptide chemicals, more preferably less than about 20% chemical precursors or non-peptide chemicals, still more preferably less than about 10% chemical precursors or non-peptide chemicals, and most preferably less than about 5% chemical precursors or non-peptide chemicals.

30 Chemical synthesis of polypeptides facilitates the incorporation of modified or unnatural amino acids, including D-amino acids and other small organic molecules. Replacement of one or more L-amino acids in a peptide with the corresponding D-amino acid isoforms can be used to

increase the resistance of peptides to enzymatic hydrolysis, and to enhance one or more properties of biologically active peptides, i.e., receptor binding, functional potency or duration of action. See, e.g., Doherty, et al., 1993. *J. Med. Chem.* 36: 2585-2594; Kirby, et al., 1993. *J. Med. Chem.* 36:3802-3808; Morita, et al., 1994. *FEBS Lett.* 353: 84-88; Wang, et al., 1993. *Int. J. Pept. Protein Res.* 42: 392-399; Fauchere and Thiunieau, 1992. *Adv. Drug Res.* 23: 127-159.

Introduction of covalent cross-links into a peptide sequence can conformationally and topographically constrain the polypeptide backbone. This strategy can be used to develop peptide analogs of the fusion polypeptides with increased potency, selectivity and stability. Because the conformational entropy of a cyclic peptide is lower than its linear counterpart, adoption of a specific conformation may occur with a smaller decrease in entropy for a cyclic analog than for an acyclic analog, thereby making the free energy for binding more favorable. 10 Macrocyclization is often accomplished by forming an amide bond between the peptide N- and C-termini, between a side chain and the N- or C-terminus [e.g., with K₃Fe(CN)₆ at pH 8.5] (Samson et al., *Endocrinology*, 137: 5182-5185 (1996)), or between two amino acid side chains. 15 See, e.g., DeGrado, *Adv Protein Chem.* 39: 51-124 (1988). Disulfide bridges are also introduced into linear sequences to reduce their flexibility. See, e.g., Rose, et al., *Adv Protein Chem.* 37: 1-109 (1985); Mosberg et al., *Biochem Biophys Res Commun.* 106: 505-512 (1982). Furthermore, the replacement of cysteine residues with penicillamine (Pen, 3-mercaptop-(D) 20 valine) has been used to increase the selectivity of some opioid-receptor interactions. Lipkowski and Carr, *Peptides: Synthesis, Structures, and Applications*, Gutte, ed., Academic Press pp. 287-320 (1995).

Pharmaceutical Compositions Including Glycoprotein Iba Fusion Polypeptides or Nucleic Acids Encoding Same

The glycoprotein Iba fusion proteins, or nucleic acid molecules encoding these fusion 25 proteins, (also referred to herein as "Therapeutics" or "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the 30 most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the

field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art.

- 5 Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The active agents disclosed herein can also be formulated as liposomes. Liposomes are prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang et al., Proc. Natl. Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. 10 Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized 15 phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), 20 transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as 25 ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

30 Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include

physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of

5 microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the

10 action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays

15 absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a glycoprotein Iba fusion protein) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle

20 that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

25 Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a

disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

5 For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated 10 are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

15 The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, 20 including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as 25 pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

In some embodiments, oral or parenteral compositions are formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to 30 physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for

the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

5 The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.*, U.S. Patent No. 5,328,470) or by stereotactic injection (*see, e.g.*, Chen, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

10 Sustained-release preparations can be prepared, if desired. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

15 The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

METHODS OF INHIBITING ADHERENCE OF IN A BIOLOGICAL SYSTEM

20 Also included in the invention are methods of inhibiting adherence of a blood cell to a biological tissue in a biological system. The method includes adding to a biological system a fusion polypeptide of the invention in an amount sufficient to inhibit adherence of a blood cell to the biological tissue.

The blood cell can be for example, a leukocyte, platelet or red blood cell. The leukocyte can be any leukocyte that is capable of adhering to a biological tissue. In various aspects the leukocyte is a granulocyte, (*i.e.*, neutrophil, basophil or eosinophil), monocyte (*i.e.*, macrophage) or lymphocyte (*e.g.*, T-lymphocyte, B-lymphocyte, tumor infiltrating lymphocytes or natural killer cell). In some embodiments, the leukocytes express a β 2 intergin, *e.g.* Mac-1. Alternately, the leukocyte expresses a selectin ligand.

Also included in the inventions are methods of inhibiting adherence of a protein to a biological tissue in a biological system. The method includes adding to a biological system a fusion polypeptide of the invention in an amount sufficient to inhibit adherence of the protein to the biological tissue.

The protein can be membrane associated (*e.g.*, covalently, non-covalently, ionicly). Alternatively, the protein can be in a soluble form (*i.e.*, in solution). The protein is von Willibrand Factor, thrombin, P-selectin or glycoprotein Iba.

As used herein a "biological tissue" is meant to include one or more cells with or without intracellular substances (*e.g.*, extracellular matrix proteins, polysaccharides and proteoglycans). A biological tissue also includes solely extracellular matrix substances, such as the subendothelium connective tissue matrix. In some aspects the biological tissue is the vascular endothelium. The biological tissue can be one or more platelets or leukocytes. In various aspects the biological tissue is complexed with a component of the GP Ib-IX-V complex such as glycoprotein Ib α , Mac-1, P-selectin, thrombin or a von Willibrand Factor. By "complexed" is meant that the biological tissue contains a soluble form of a component of the GP Ib-IX-V complex. Alternatively, "complexed" is meant that the biological tissue contains a cell that expresses a component of the GP Ib-IX-V complex.

As used herein a biological system is meant to include any system that comprises biological components, *e.g.*, cells, proteins, carbohydrates, lipids or nucleic acids. The biological system can be an *in vivo*, *ex vivo* or *in vitro* system.

By "adherence" is meant to include any leukocyte- biological interaction, *e.g.*, rolling, firm attachments or specific interaction.

Inhibition of adherence of a blood cell or protein to a biological tissue can be measured using methods known in the art. For example, assays for detecting binding of glycoprotein Iba to a biological tissue are described in Simon et al., J. Exp. Med. 192:193-204, 2000, and references cited therein. In various embodiments, binding of a GP Ib α fusion protein inhibits

binding of a blood cell or protein to a biological tissue by at least 30%, 50%, 75%, 90%, 95%, 99% or 99.9%.

Adherence can also be assessed in condition of greater or less than physiological flow conditions, including static conditions and serial application of static and shear conditions.

- 5 Adherence can be determined for example colormetrically, flurometrically, by flow cytometry or using a parallel plate flow chamber assay.

Also included in the invention are methods of treating platelet activation associated disorders in a subject by administering to a subject a biologically-active therapeutic compound (hereinafter "Therapeutic"). Alternatively, the subject is also administered one or more of the following 10 acetylsalicylic acid, e.g., aspirin heparin, e.g., unfractionated or low-molecular weight heparins, glycoprotein IIb/IIIa antagonists, clopidogrel, P-selectin antagonists, thrombin inhibitors or thrombolytic enzymes.

The subject can be e.g., any mammal, e.g., a human, a primate, mouse, rat, dog, cat, cow, horse, pig.

- 15 The Therapeutics include, e.g.: (i) any one or more of the glycoprotein Ib α fusion polypeptides, and derivative, fragments, analogs and homologs thereof; (ii) antibodies directed against the glycoprotein Ib α fusion polypeptides; and (iii) nucleic acids encoding a glycoprotein Ib α fusion polypeptide, and derivatives, fragments, analogs and homologs thereof.

Essentially, any disorder, which is etiologically linked to platelet activation, is considered 20 amenable to prevention or to treatment. The disorder can be, e.g., vascular inflammation, atherosclerosis, restenosis (e.g., angioplasty-related restenosis) and/or a condition associated with thrombotic disease, e.g., angina, (i.e., stable angina and unstable angia) acute myocardial infarction, stroke, venous thrombosis or arterial thrombosis.

The invention will be further illustrated in the following non-limiting examples.

25 **EXAMPLE 1: PRODUCTION AND PURIFICATION OF RECOMBINANT GP1B-IG FUSION PROTEINS**

Three GP1b-Ig fusion proteins, GP1b302-Ig (SEQ ID NO:1), GP1b290 Ig (SEQ ID NO:4), and GP1b290/2V-Ig (SEQ ID NO:5), were produced by recombinant methods and purified. Chinese hamster ovary (CHO) cells lacking dihydrofolate reductase (DHFR) activity were stability transfected with linearized plasmid DNA consisting of a mammalian expression 30 vector directing the transcription of a GP1b-Ig coding regions in polycistronic fashion with a DHFR selectable marker gene. Candidate expressing cells were selected in medium containing

increasing concentrations of methotrexate (MTX) essentially as described in Kaufman et al. Nucleic Acids Res. (1991) 19:4485-90. For collection of GP1b-Ig conditioned medium, CHO cells were grown to near confluent levels on 5-20 culture dishes (150mm diameter), the cell monolayer was washed twice with PBS and cells were cultured for approximately 24hrs in 5 medium lacking fetal bovine serum. The medium was then collected and cells discarded.

CHO cell condition media (CM) was adjusted to 50 mM Tris pH8.0, 200 mM NaCl, filtered through a 0.2 um filter, and applied to a Poros Protein A column. The column was washed with 10 column volumes of 50 mM Tris pH 8.0, 200 mM NaCl and eluted with Pierce IgG elution buffer. The protein peak was followed by absorbance at 280 nM. The pH of the 10 elute was adjusted with 0.1 volumes of 1 M Tris, pH 8.0. The protein was then concentrated and the buffer exchanged by finger dialysis (25kD MWCO) against TBS (10 mM Tris, pH 8.0, 150 mM NaCl). The concentrated protein was then further purified by gel filtration chromatography on a TosoHaas G3000SW column run in TBS.

The purified protein was analyzed by Western Blots. Briefly, 13 microliters of CHO cell 15 conditioned medium was loaded per lane on a 4-20% reducing SDS PAGE gel. Western transfer was performed using Electroblot apparatus and nitrocellulose membrane (Novex, San Diego, CA). The primary detection antibody was monoclonal API, and secondary antibody was an HRP-conjugated goat anti-murine IgG (GTI, Brookfield, WI). HRP detection was via ECL system (Amersham-Pharmacia Biotech).

20 **EXAMPLE 2: IN VITRO INHIBITION OF PLATELET AGGREGATION**

The ability of the glycoprotein Ib α polypeptide-immunoglobulin fusion polypeptide to inhibit platelet aggregation *in vitro*, was determined. Platelet rich plasma (PRP) from freshly drawn, citrate blood was prepared by differential centrifugation for 10minutes at 900rpm. 0.4mls of PRP (3×10^8 /ml) was preincubated for 5 minutes at 37°C with various concentrations of 25 GP1b290/2v-Ig. Ristocetin was added to 1.5mg/ml to induce platelet aggregation. Aggregation was measured using a Sienco DP247E aggregometer. Aggregation was quantified and recorded on a chart recorder by monitoring the increase in light transmittance with stirring at 1000 rpm. As illustrated in Figure 4, GP1b290/2v-Ig inhibited ristocetin induced platelet aggregation.

EXAMPLE 3: IN VIVO INHIBITION OF REPETITIVE CORONARY ARTERY THROMBOSIS

The ability of a glycoprotein Iba GPIb290/2V-Ig polypeptide-immunoglobulin fusion polypeptide to inhibit coronary artery thrombosis *in vivo* was determined using the procedure described by Folts et al., Circulation 54:365-70, 1976.

5 Mongrel dogs, weighing 20-25kg, were anesthetized with sodium pentobarbital (30mg/kg i.v.), then intubated and ventilated with room air using a respirator. Venous and arterial catheters were placed. The heart was approached by left thoracotomy through the fifth intercostal space. The pericardium was opened and sutured to the wound edges to provide a cradle without displacing the heart. About 2cm of the left circumflex coronary artery (LCX) was isolated. Mean
10 and dynamic LCX flow was continuously monitored using a perivascular ultrasonic flow probe placed proximally on the artery. After a stabilization period, the endothelium of the LCX was injured by squeezing with a hemostat. A plastic constrictor was placed distal and overlying the area of injured endothelium to provide approximately 70-80% vessel stenosis. When blood flow decreased to zero, the blood flow was restored by shaking the constrictor to dislodge aggregated
15 platelets. This decrease and restoration of blood flow are termed CFRs. At least five consecutive CFRs were recorded prior to administering the test drug.

Representative results are shown in FIG. 5. The tracings indicate that increasing amounts of glycoprotein Iba GPIb290/2V-Ig resulted in higher blood flow. These results demonstrate that glycoprotein Iba GPIb290/2V-Ig inhibits thrombosis in the animal model..

20 A schematic illustration depicting an injured coronary artery with high fluid shear blood flow is presented in FIG. 6. The figure depicts an injured coronary artery with high fluid shear blood flow. The vessel has a segment of damaged endothelium that exposes subendothelial matrix proteins, including immobilized vWF. In the presence of GP1b alpha fusion polypeptide (GPIb-Ig), the vWF binding site is blocked, thereby preventing platelet adherence via the
25 platelet-bound GPIb alpha within the GPIb-V-IX complex. Lukocyte capture is also diminished.

OTHER EMBODIMENTS

While the invention has been described in conjunction with the detailed description
30 thereof, the foregoing description is intended to illustrate and not limit the scope of the invention.

which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.